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Ebola Virus Disease, Diagnostics and Therapeutics: Where is the Consensus in Over Three Decades of Clinical Research?

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ABSTRACT

Ebola virus is a neglected tropical disease widely accepted as one of the most fatal diseases of human and nonhuman primates ever encountered in recent years. For a long time, development of vaccines and anti-Ebola drugs has been slow. However, with recent devastating outbreaks in West Africa and Democratic Republic of Congo, the development of rapid diagnostic methods, vaccines, and antiviral drugs are at different stages of clinical trials. Despite the progress made in fighting the Ebola virus epidemic, the approaches still face various obstacles. Such obstacles highlight the desire to search for effective diagnostic and therapeutic interventions. Furthermore, Ebola virus is associated with adverse indirect effects since resources are diverted from programs aimed at controlling important diseases such as malaria, tuberculosis, and HIV infection. We hypothesize that a significant burden of Ebola viral disease if undetected may lead to missed opportunities for prevention and heighten the risk for large-scale outbreaks and pandemics. Further, Ebola is associated with high fatality rate and significant socio-economic impact. Therefore, the need for a rapid diagnostic technique to be used at the point of care, universal Polyvalent-*Ebolavirus* vaccine, and effective anti-Ebola drug cannot be overemphasized. Thus, this review focuses on the availability, suitability, and significance of current advances in diagnostics, vaccines, and therapeutic options for the Ebola disease and the need to develop novel vaccines and antiviral therapies that are effective against all known Ebola virus species.

Introduction

Worldwide transmission of emerging and re-emerging viral infections that can overwhelm public health resources such as Ebola virus disease is a threat to human health. The first incidence of Ebola virus disease in humans was reported in South Sudan in 1976 [20], although it is believed to have occurred as early as 1972 in the Democratic Republic of Congo [40]. Ever since it is widely accepted that the Ebola virus disease is among the most fatal diseases of human and non-human primates ever encountered [93]. The disease is considered among the top five most dangerous diseases, as captured in the

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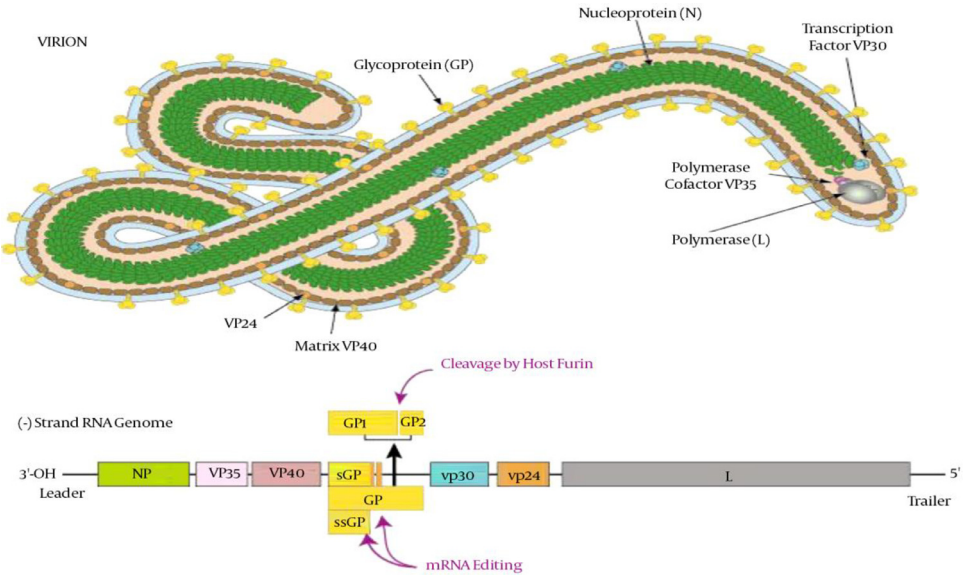

Fig. 1. Illustration of the structure and genome of the Ebola virus, Adopted from Nature and History of Ebola Virus by Majid et al. [54].

list of prioritized diseases [93]. The significance of Ebola hemorrhagic fever lies on its morbidity [13], the splanchnic manner in which it kills, the high mortality rate [46] and absence of efficacious drugs [93]. As such, Ebola virus disease has become a global public health burden that has led to major epidemics in resource-poor settings and posing an imminent threat of global pandemics [46]. Thus, the Ebola virus is identified as a bio-safety level-4 pathogen and CDC category A-agents of bioterrorism [14].

Ebola virus disease outbreaks largely been restricted to sub-Saharan Africa [55]. For instance, the current complex outbreak in the Eastern Democratic Republic of Congo in which 2273 people have died out of 3453 [94,96]. Similarly, the infamous 2014–16 West African infection which was the largest and the most devastating, recording over 28,000 infections and more than 11,000 fatalities [15]. Every Ebola virus outbreak presents a serious global danger due to the potential of cross-border spillover and the risk of an Ebola pandemic [94]. As such, an Ebola Virus disease is more often considered a Public Health burden of International Concern due to its potential of international spread [92,95]. Indeed secondary infection following patients traveling from affected African countries was reported in the USA, Italy, Spain, and United Kingdom [15,49]. The unfamiliarity with the Ebola virus disease outside the endemic areas usually leads to delayed diagnosis and management response [49] thus precipitating the spread of Ebola virus disease in the population.

Ebolavirus

Ebola Virus Disease is a complex zoonotic disease of human and nonhuman primates [27]. The virus belongs to the genus *Ebolavirus* of the family *Filoviridae* [8]. The genus contains six known species: *Reston ebolavirus* (Reston virus), *Sudan ebolavirus* (Sudan virus), *Zaire ebolavirus* (Ebola virus), *Bundibugyo ebolavirus* (Bundibugyo virus), *Tai Forest ebolavirus* (Tai Forest virus) [8] and the recently identified *Bombali ebolavirus* (Bombali virus) [25]. Although *Bombali ebolavirus* has been isolated from bats in Sierra Leone and Kenya, it has not been associated with any human or nonhuman primate infections [25]. All of the above Ebola viruses are endemic in Africa except for the Reston virus that is localized in Asia [76]. Of all, the six *Ebolavirus* species, *Zaire ebolavirus*, and *Sudan ebolavirus* are the most lethal with high fatality amongst humans and non-human primates while the Reston virus are mostly associated with non-human infections [22]. The overall mortality Ebolaviridae is 25%, 50%, and 80% for Bundibugyo virus disease, Sudan virus disease and Ebola virus disease (Zaire ebolavirus) respectively [6].

Structures and functional characteristic of Ebola virus

Ebola virus is a negative-sense single-stranded RNA ((-) ssRNA) with a 19 kb genome [55] that infect the macrophages and dendrite cells [56]. Ebola virus genome is 18,959 nucleotides in length and 80 nm in width. The viral genome contains only 7 open reading frames (ORFs) with limited encoding capacity (Fig. 1). Thus the virus expands its gene function by assigning more roles to each of them. As such, nine proteins which include glycoprotein (GP), soluble glycoprotein (sGP), small soluble glycoprotein (ssGP), nucleoprotein (NP), the polymerase cofactor viral protein (VP35), the major matrix protein (VP40), the minor matrix protein (VP24), transcription activator (VP30) and Viral RNA-dependent RNA polymerase (*L*) are known to be translated by the limited viral genome [2]. Structural proteins that are responsible for replication and transcription of viral RNA are NP, VP35, VP30 and L proteins, while those responsible for assembly, budding, and release of virion

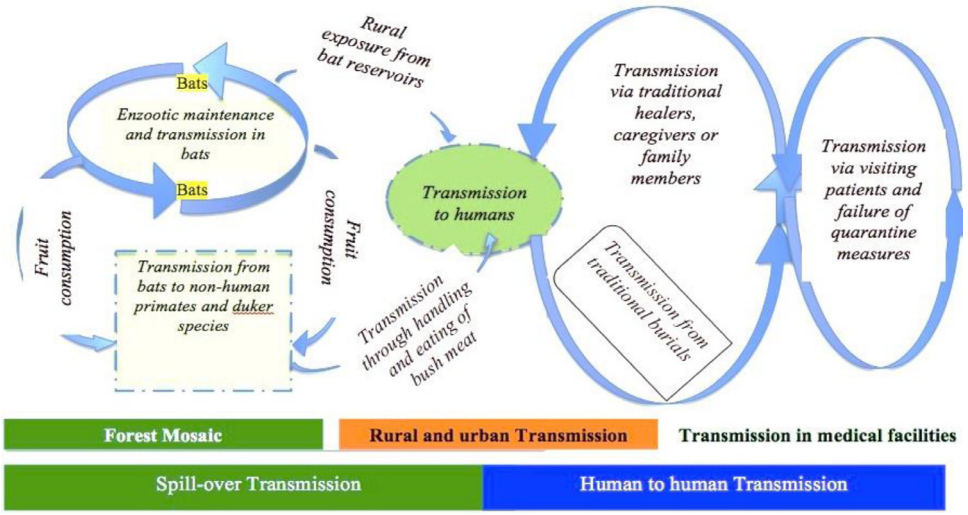

Fig. 2. A Schematic illustration summarizing Ebola virus transmission at the wildlife-human interface and human-to-human transmission; Ebola spillover to humans from wildlife is associated with hunting and direct contact with body fluids and tissues of infected animals, for example, the primates, fruit bats and duikers [3,46]. An outbreak in wild chimpanzees is associated with predation of infected red colobus monkey [26]. Bats may mechanically infected animals e.g. duiker, nonhuman primates, and humans who consume fruits contaminated with Ebola-infected bat fecal matter or saliva. Furthermore, Human-to-human transmission is associated with direct contact with infected people or their bodily fluids, caregiving to the people suffering from Ebola disease, and traditional burial [55]. Transmission in high-density urban centers and lastly transmission in hospitals associated with failures in infection control procedures and isolation precautions associated with inadequate staffing, lack of infrastructure, and financing of health care systems [92]. *Modified from, what factors might have led to the emergence of Ebola in West Africa by* Alexander et al. (2015) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

particles includes VP40 and VP24. Besides, VP35 and VP24 are involved in immune evasion by blocking interferon production and signaling [1]. The surface GP is responsible for cellular attachment, fusion, cell entry, and evasion of the immune response [80]. Therefore, the functional characterization of the viral GP makes it an important antigen target for designing Ebola vaccines and immunotherapies.

Epidemiology of the disease

The onset and spread of Ebola are attributed to an initial spillover infection with an individual coming into contact with the body fluids of an infected animal [46], more likely a reservoir fruit bats or infected chimpanzee [3]. Secondary human transmission is through direct contact with body fluids from infected people [27]. Also, caring for the sick or handling dead bodies is associated with high risk which explains the occurrence of nosocomial transmission before the outbreak is identified (Fig. 2) [55]. Sexual transmission from EBOV survivors [59] and the risk of aerosol transmission has also been reported [4]. With the significant burden and public health challenges currently posed by the disease, Ebola virus has stimulated active research in diagnostic, vaccine development, and therapeutic options [61,89].

Economic burden of Ebola virus disease

Although the human costs of an Ebola outbreak due to direct fatalities are well understood [15], the economic impact is largely overshadowed by the overall mortalities. However, Ebola epidemic may lead to pronounced socio-economic impact. According to World Bank projection, Guinea, Liberia, and Sierra Leone lost up to 2.2 billion US dollars by 2015 in their gross domestic product. The heavily affected sectors were the private sector, agricultural production, and cross-border trade [15]. Furthermore, the outbreaks adversely affect the community health since resources were diverted from programs aimed at controlling other diseases including malaria, tuberculosis, HIV infection [65] and trypanosomiasis [12]. Besides, epidemiological mapping of previous outbreaks and reservoir habitat has put a population of 22 million at risk of Ebola virus infection in Africa [67]. Concerning this, the lack of known therapeutic measures, the high fatality rates and the significance of socio-economic impact by the disease, the need for a novel therapeutic option and a universal polyvalent-vaccine to be used in preventing the spread of the disease cannot be overemphasized.

The role of limited detection, prevention and treatment capacities on Ebola outbreak

Ebola virus disease is one of the highly severe and fatal diseases of primates with great economic losses. Lack of access to rapid Ebola virus diagnostic tools in both underdeveloped and developed regions has proven to be a setback to early identification, isolation, and management of reported cases as well as public health response [78]. The non-specificity of symptoms

presented during early stages of Ebola disease infection such as fever, headache, and myalgia are non-pathognomonic and makes early detection of Ebola virus outbreak a challenge [15,36]. With such nonspecific symptoms and Ebola virus being a highly contagious disease [13], the disease can be spread uncontrollably before it is diagnosed and public health control mechanism set in place, Indeed studies from previous Ebola virus infections in West Africa [15] and Democratic Republic of Congo [94,96] demonstrated that limited availability of rapid diagnostic assays in developed regions did result in delays in differential diagnosis of Ebola virus disease from other hemorrhagic diseases among returning travellers [[15],[49]] therefore contributing to the initial failure of managing and containing the spread of the disease.

With limited diagnostics, lack of point of care diagnostic tools, and the high morbidity and mortality associated with Ebola virus disease [[92],[94]], vaccine and therapeutics development are critical in achieving long-term containment of Ebola virus disease. Indeed, vaccine development has been of outmost research priority ever since the first Ebola virus disease was reported [98]. Several candidate vaccines developed are in different stages of clinical trials. Similarly, advances in research have developed a number of experimental drugs against Ebola virus disease with a few like REGN-EB3 and mAb114 showing promising results [[61],[94]]. However, 50 years after the first Ebola virus disease outbreak was reported [40], there are no licensed vaccines [62] or therapeutic [[61],[94]] option to be used in prevention of Ebola virus disease.

It is important to note that lack of rapid diagnostic methods, unavailability of a licensed vaccine and anti-Ebola drugs to be used in prevention and management of the disease, has proved to exacerbated the spread of the disease during outbreaks. Considering the 2014-2016 Ebola virus disease outbreak in West Africa, it is arguably agreed that the economic burden of Ebola virus disease is largely overshadowed by the overall mortalities that occur during an outbreak [15]. Studies have shown that, if the intervention had been instigated early enough West Africa outbreak, 8835 deaths would have been averted and up to 374 million US$ would have been saved [[12],[15],[44],[65]]. As such, Ebola epidemic may lead to pronounced socio-economic impact. It is therefore imperative to say that the importance of early detection and surveillance of the disease, availability of vaccines and therapeutic options, especially in the limited resource African countries, where the disease is endemic cannot be over emphasized.

Way forward

Detection and diagnosis

Early and timely diagnosis and detection of Ebola virus disease is key in combating and managing the spread of the Ebola virus outbreak. However, Ebola virus disease diagnosis shortly after an infection is difficult since the early symptoms of the disease such as fever, headache, and weakness are non-specific to Ebola disease and are associated with a more common disease like typhoid and malaria [15]. Although many diagnostic techniques are available, most of them take time and cannot be employed at the point of care for the rapid diagnosis of the disease. The available diagnosis methods are discussed below.

Cell culture

Cell culture technique has been used as the gold standard method for confirming the presence of the Ebola virus disease. The technique is routinely used in biosafety level-4 containment research and public health laboratories for isolation and propagation of Ebola virus in Vero E6 cell culture. The propagated viral culture is then visualized by electron microscopy [5]. Cell cultures are relatively unbiased and are critical in identification of the causative agent. However, cell cultures as a few limitations such as, cell culture is considerable slow and takes time for the results to be availed during which the disease may have spread through the population. In addition, cell culture technique is also restricted by the ability of the virus to grow only on a particular cell line and must be carried out in a specialized laboratories to avoid risk of infections [32].

Serological assays

Serological assays are routinely used in research and public health laboratories for the detection of specific antibodies against the Ebola virus in patient serum. Serological assays have played a critical roll in demonstration of prior or current infections of Ebola virus disease [[34],[42]].

Two serological assays, the indirect fluorescent antibody detection test (IFAT) [42] and enzyme-linked immunosorbent assay (ELISA) [48] have been used in the detection of Ebola virus antibodies. Indirect fluorescent antibody detection played a significant role in distinguishing the Ebola virus from the Marburg virus during the 1976 Ebola outbreak [42]. In IFAT, either cell cultures infected with Ebola virus disease or antigen suspensions from these cell cultures are irradiated, fixed on a slide, and incubated with serum from Ebola virus exposed individuals. The bound antibodies are then detected with a fluorescent-labeled rabbit anti-human IgG antibody and visualized with a fluorescent microscope [99]. On the other hand, ELISA utilizes Ebola virus antigens prepared from cell cultures inoculation that binds the Ebola virus-specific IgG and IgM antibodies in patient serum [48]. The IgM ELISA in use entails an antibody capture platform that utilizes microtiter plates coated with goat antibodies that bind to human IgM present in sera samples. The Ebola virus-specific IgM is detected by incubation of the microtiter plates with prepared Ebola virus antigens. A polyclonal antibody that has been isolated from a rabbit exposed to the Ebola virus is then used. Finally, horseradish peroxidase-conjugated anti-rabbit antibodies mediate the detection. Similarly, the IgG ELISA utilizes plates coated with Ebola virus antigens that bind to Ebola virus-specific antibodies present in sera samples. The horseradish peroxidase-conjugated mouse antibodies mediate detection of the viral-specific IgG antibody

[19]. Besides, it is demonstrated that IgG antibodies react with antigens from multiple Ebola virus species as compared to the minimally cross-reactive IgM [53]. Given the existing challenges in generating viral antigens from cultures, ELISA that effectively uses a recombinant viral protein [75] and a monoclonal antibody against nucleoprotein [63] has been developed. However, they are yet to be validated for clinical use. Unlike IFAT that is considered to be non-specific and less sensitive, thus unsuitable for large-scale diagnostics, ELISA offered a reliable, rapid, and high-throughput system for the Ebola virus serological test that can easily be employed for a large-scale diagnostic effort. Despite the above advantages, ELISA as most serological assays has certain limitations. First, the onset of both IgM and IgG antibody response is between 6 and 11 days for IgM and 9 to 11 days for IgG antibodies after the onset of symptoms [[48],[88]] thus, causing delays in therapeutic response that leads to fatal outcomes. Secondly, the procedure is time-consuming, laboratory-based, requires trained personnel and specialized equipment [17]. Finally, given the high morbidity and mortality of the Ebola virus disease, handling of the virus and suspected samples requires BSL-4 containment laboratory [14]. With all these limitations, serological assays, including ELISA, cannot be applied in early detection or at the point-of-care diagnosis of Ebola virus disease. However, it provides a useful tool for a population level seroprevalence studies [48].

Protein antigen detection assay

Protein antigen detection of Ebola virus protein circulating in the blood is a considerably reliable and rapid diagnostic method for detection of acute Ebola virus disease, as viral proteins increase to detectable levels within a few days of disease onset [[47],[63]]. Protein antigen detection is an ELISA platform that utilizes 8 monoclonal mouse antibodies and polyclonal antibodies from hyperimmune rabbit serum reactive against Zaire, Sudan, and Reston Ebola viruses [47]. Protein antigen detection is reliable and a fast way of detecting viral antigen in serum [85]. This method was significant in the clinical diagnosis of Ebola virus disease during the 1995 outbreak in DRC [48] and 2000 outbreak in Uganda [85]. As observed, events in early Ebola virus infection including early detection determine disease progress, recovery, or eventual death [15]. Furthermore, protein antigen detection proved to be a reliable method to indicate active infection since antigen levels rise throughout the disease after which, they decline to undetectable levels by day 16 [85]. As such, the need to analyze the spatial-temporal distribution of Ebola virus antigen expression and the promising cross-reactivity of human antibodies against Ebola virus in the development of rapid serological tests to be used at the point-of-care cannot be overemphasized. Currently, Ebola virus diagnostic protocol recommends the use of transcriptomic approaches more so RT-PCR as the preferred diagnostic method [[16],[31],[85]].

Transcriptomic approaches

Given the severity and high mortality associated with the Ebola virus disease, rapid and more accurate detection of this disease is essential both for controlling and preventing further transmission [74]. These challenges facing detection of Ebola virus disease has been circumvented by molecular-based diagnostic methods that employ the use of reverse-transcription polymerase chain reaction [[16],[31],[85]]. Reverse Transcription-Polymerase Chain Reaction is considerably the preferred diagnostic method and is used as the standard to quantify viral infection in clinical settings [16]. In these assays PCR is used to amplify the GP, NP, VP35, VP40, VP30, VP24 and L genes, followed by size based amplicon detection via either gel electrophoresis or in real-time via Real-time PCR [86]. The RT-PCR protocols involve sample collection and inactivation, extraction of Ebola virus RNA, reverse transcription and cDNA amplification [[66],[86]]. Development of viral inactivation buffer, guanidinium thiocyanate, as part of RT-PCR RNA extraction kits [66] has revolutionized the handling of Ebola virus samples as samples may be processed in a remotely established field laboratory. Besides, the integration of Real-time (quantification) Reverse Transcription Polymerase Chain reaction (RT-qPCR) has allowed for direct quantification of the Ebola virus RNA [75]. The RT-qPCR technique utilizes a florigenic probe such as Tag-man systems [[66],[86]] and the intercalating fluorophores such as the SYBR green 1 [75].

Despite the several advantages of the RT-PCR diagnostic platform, the PCR platform is associated with certain limitations. First, it is appreciated that working with RNA is quite tiresome and requires the storage of temperatures between -70 °C and -80 °C to prevent RNA degradation. Similarly, proper handling of the sample between extraction and RT-PCR steps is very critical because sample contamination risks having a false-positive result [85]. As such, handling and processing of the samples require utmost care and trained personnel, hence cannot readily be incorporated into a routine healthcare diagnostic laboratory. Secondly, PCR amplification depends on thermal cycling involving denaturation of cDNA, primers annealing to cDNA and amplification of cDNA, as such RT-PCR procedures require a constant electric power supply [33] thus; deployment of PCR-based diagnostic methods to remote field laboratory is limited by the availability of reliable electric power supply. Thirdly, advances in miniaturization of thermo-cycler platforms seek to increase sensitivity, reduce sample and reagent requirement, speed the turnaround time and allow for portability and field deployment [45]. However, designing a Lab-on-a-chip for nucleic acid-based Ebola virus diagnostics requires an elaborate chip infrastructure to run the different thermal cycling processes and enabling of separation of reagents on a small scale for high throughput as reviewed by Coarsey et al., [17]. Fourthly, the genetic drift associated with the viral RNA-dependent DNA polymerase enzyme. For instance, the glycoprotein (GP) is associated with an average mutation rate of 3.6×10^{-5} substitutions per site per year [83]. The recent 2014–2016 indicated an increased mutation rate of 8×10^{-4} site substitutions per year [31]. These specific site mutations alter the viral genome sequences thus reducing the specificity of the RT-PCR primers. The reduced specificity means that, with every outbreak, there is reduced sensitivity since the primers become less sensitive to the mutated viral genome [31]. Therefore, genomic drift due to accumulated point mutation may influence false-negative and false-positive

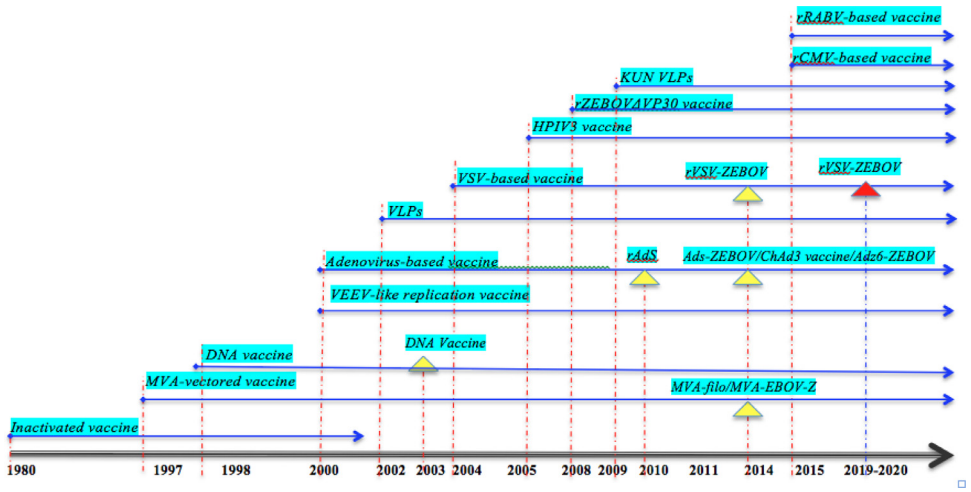

Fig. 3. A Schematic illustration summarizing the Ebola vaccine development, each continuous blue line is a representation of the development of each kind of vaccine and its name. The yellow triangle is the start date of the candidate vaccine clinical trials, while the red triangle indicates the license date for commercial production. In 2002, research on the inactivated vaccine was retired. *Modified from Ebola vaccines in a clinical trial: The promising candidate by* Wang et al. [89] (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

results [79]. Thus, to circumvent the challenges caused by the viral genome shift and for accurate identification of emerging Ebola strains, the need to develop a large number of specific primes to be used for accurate diagnosis during an outbreak cannot be overemphasized.

Development of vaccines

To the best of our knowledge, there are currently no commercially available vaccines to prevent Ebola virus disease. However, there has been accelerated development of an Ebola preventive vaccine. One of the most promising vaccines that are in the process of being licensed is the genetically engineered recombinant vesicular stomatitis virus-Zaire Ebola virus (rVSV-ZEBOV) [[37],[94]].

The development of vaccines and treatment options for Ebola virus disease has been a research priority ever since the first case of the disease was reported as mentioned in Wu et al., [98]. The currently available vaccine platforms range from non-replicating and replication-competent viral vectors to DNA and subunit vaccines (Fig. 3).

Developments of inactivated virus and subunit vaccines

An inactivated virus of the Zaire Ebola virus and classical subunit vaccines were the first to be tried against the Ebola virus disease. The initial inactivated viral vaccine against the Zaire Ebola virus was highly effective in rodent models but ineffective to nonhuman primates models [30]. Subunit vaccines made from recombinant purified viral proteins against Zaire Ebola virus disease that was later introduced proved to have low immunogenicity and were less protective in rodents as reviewed by Hoenen *et al.,* [41]. Research on these types of vaccines was retired in 2002.

Protein-based virus-like particle vaccines

The introduction of protein-based vaccines that contain virus-like particles proved to be highly effective in rodents and to nonhuman primate models [90]. The virus-like particle contained Zaire Ebola virus matrix protein (VP40), glycoprotein (GP), or containing the nucleoprotein (NP) [90]. The virus-like particles are known to be safe and highly immunogenic since it induces humoral, innate and cellular immune responses in both rodents and nonhuman primates [91]. Further, a combination of Zaire Ebola virus GP and Marburg virus VP40 protein particles was demonstrated to offer cross-protection against Zaire Ebola virus and Marburg virus diseases in rodents model [84]. However, the cross-protection and the safety of the combined ZEBOV-GP-MARV-NP virus-like particles vaccines in nonhuman primates and humans are yet to be investigated [89].

Developments of non-replicating vaccine vectors

Another important platform that has revolutionized the development of the Ebola disease vaccine is the use of non-replicating vaccine vectors. In this platform, the Venezuelan equine encephalitis virus was used in the development of the Ebola virus vaccine development. In this platform the structural genes of attenuated Venezuelan equine encephalitis virus were replaced with Zaire Ebola virus or Sudan Ebola virus -GP, -NP, -VP24, -VP35 or -VP40 [[39],[97]]. These vector-based vaccines proved to be highly immunogenic. As reported, those vector-based vaccines expressing -NP and -GP conferred 100% protection in rodents [97] and nonhuman primate models [39]. Beside the use of Venezuelan equine encephalitis virus as a

vector, the Kunjin virus has also been used in developing the ZEBOV-GP vaccine that proved to be effective in rodent models [71].

Development of DNA vaccine

A DNA vaccine is an important platform with several inherent advantages such as DNA can adapt rapidly as the pathogen evolve, they can be produced in large quantities using the non-infectious plasmids, it cannot be affected by pre-existing immunity and it induces both cellular and humoral immunity. However, it requires a multi-administration of doses to achieve desired immunity [51]. Three to four doses of Plasmid DNA encoding either ZEBOV-GP or ZEBOV-NP has been demonstrated to be highly effective in rodent models [70]. Further, analysis of a polyvalent-filovirus DNA vaccine against the *Zaire ebolavirus* (ZEBOV) *Marburg marburgvirus* (MARV) and *Sudan ebolavirus* (SUDV) in mice proved to be highly potent and offered complete protection against ZEBOV and MARV [77]. However, promising the DNA vaccine is, there are limited data on their potency in nonhuman primates [70]. There are several DNA vaccines in various stages of development and they include:

Recombinant adenovirus-based vector vaccine platform. A milestone was achieved with the introduction of a highly immunogenic recombinant Adenovirus 5 (rAd5)-based vector expressing ZEBOV antigens. The use of non-replicating recombinant adenovirus is safe and highly immunogenic thus providing desirable protection to the host [82]. Even though the initial vaccine expressing rAd5/ZEBOV-GP required a slightly higher dose to produce the desired effect, an improved rAd5 vaccine expressing interferon α (rAd5/IFNα) was highly effective at lower doses and protect 100% of immunized rodents [73] and nonhuman models [72]. As such, the Ad5-EBOV vaccine was allowed to proceed to human clinical trials. In so far, observations from clinical trials suggest that Ad5-based Ebola vaccines are safe to humans and they rapidly elicit both cellular and humoral immunity [89]. However, the magnitude of both humoral and cell response is affected by pre-existing rAd5 immunity, such that pre-existing immunity significantly lowers the protective efficacy of Ad5-based vaccines [[72],[89]]. The good news is that delivery of vaccines via either nasal, oral or intra-tracheal routes ([68]; Richardson et al., 2011) and admission of booster doses [68] not only circumvent pre-existing immunity without affecting the protective effect of Ad5-based EBOV vaccines but also significantly improves the cellular immune response. Furthermore, the change of vector backbone from rAd5 to those with no human immunity such as rAd26 and rAd35 may also circumvent the challenge of pre-existing immunity [68]. The rAd26 and rAd35 backbones as also been used to successfully develop a pan-filovirus vaccine that induced cross-reactive antibodies across multiple filoviruses [68]. For instance, when rAd5-expressing ZEBOV-GP and SEBOV-GP was used, it gave a cross-protection against Zaire and Bundibugyo Ebola viruses [38]. Although these results show that there is light at the end of the tunnel, since it is possible to develop a polyvalent-filovirus vaccine to achieve cross-protective immunity against several filovirus species, recombinant adenovirus expressing polyvalent filovirus-GP antigens vaccine against all the species of Ebola virus is yet to be developed.

Recombinant ZEBOVΔVP30 vaccine platform. The development of reverse genetics systems for RNA viruses such as recombinant ZEBOVΔVP30 led to the development of diverse variants of Ebola viruses. Development of rZEBOVΔVP30 vaccines involves the deletion of the virus-specific transcription activator (VP30) hence curtailing the ability of production and spread of viral progeny [35]. These recombinant vaccines were evaluated to be safe and offered 100% protection in both mice and guinea pig model [35]. Although the recombinant rZEBOVΔVP30 proved to be one of the interesting vaccine candidates, their efficacy in nonhuman primate's models is currently not available. Furthermore, the recombinant ZEBOVΔVP30 vaccine platform raises a lot of concerns since the virus is over 95% identical to the highly virulent wild-type Zaire Ebola virus, only lacking the essential VP30 encoding genes; nonetheless, there is no evidence for recombination events that can lead to re-integration of VP30 into the viral genome during Ebola virus replication.

Developments of replication-competent vaccine vectors

The replication-competent vaccine vectors include several recombinant vaccines platforms such as recombinant vaccinia virus-based vaccine, recombinant cytomegalovirus-based vaccine, recombinant paramyxovirus-based vaccine, recombinant vesicular stomatitis virus-based vaccine and recombinant rabies virus-based vaccine [[9]–[11],[21],[30],[58],[60],[69],[87]].

Vaccinia virus-based vaccine platform. Although currently not in development, the previous Vaccinia virus-based vaccines expressing single antigens (GP, soluble GP (sGP), NP, the polymerase co-factor VP35 and VP40) chosen from ZEBOV genes largely produced poor results. Only vaccinia virus expressing GP showed partial protection in rodent models, but could not confer protection to nonhuman primates [30]. Nonetheless, other recombinant viral-based vaccines are churning out encouraging results.

Recombinant cytomegalovirus-based vaccine platform. Recombinant Cytomegalovirus-based vaccine vector is an important replication-competent vaccine vector developed primarily to immunize African wildlife. The Cytomegalovirus was redesigned to express a cytotoxic T lymphocyte (CTL) epitope located on ZEBOV-NP. As much as the CMV-based vaccine may be ideal in achieving high vaccine coverage through the target population because of its unique potential of re-infection and dissemination, the vaccine did not offer full protection against Zaire Ebola virus disease in mice models [87]. However, analysis of Rhesus CMC expressing ZEBOV-GP has been demonstrated to offer protective immunity to macaques [58]. This platform may

revolutionize vaccine dissemination in wild-reservoir animals due to the potential of CMV vaccines to re-infect and freely disseminate in the population providing persistent immune protection within the population. However, there is need to further investigate immunity development following animal-to-animal spread of the vector and the suitability of this platform as a vector for human vaccine dissemination.

Recombinant paramyxovirus-based vaccine platform. The recombinant Paramyxovirus-based vaccine platform is still under development. It involves the use of negative-stranded RNA, specifically the use of the human parainfluenza virus 3. Human parainfluenza virus 3 is a common respiratory pathogen and is highly investigated as a vaccine vector in measles infection [21]. A modified recombinant HPIV3 expressing Zaire Ebola virus –GP or –NP has been demonstrated to offer 100% protection to rodent models [10] and to rhesus macaques monkey [9]. To overcome the pre-existing immunity against HPIV3, the vector had to be improved by deleting HPIV3 *F* and *HN* genes. The improved rHPIV3ΔFΔHN/ZEBOV-GP proofed to be more efficient and gave desired protection to rodent models against the Zaire Ebola virus challenge [11]. For some time, research of the recombinant HPIV3 vaccine vector slowed down, but the infamous 2014–2016 West African Ebola infection prompted increased vaccine development. As such, an attenuated recombinant human parainfluenza virus type 1 expressing EBOV-GP was designed and its immunogenicity investigated. The rHPIV1/EBOV-GP elicited high levels of neutralizing antibodies and desired protection in monkey models [50]. The potential for needle-free administration, the relative ease of large-scale production and the ability to induce systemic and local immunity following an intranasal administration are some of the advantages that makes attenuated recombinant human parainfluenza virus vaccine platform suitable for designing candidate vaccine [7]. However, their efficacy to instigate immunogenic response and if a pre-existing parainfluenza immunity reduces its efficacy needs to be determined.

Recombinant vesicular stomatitis virus-based vaccine platform. A recombinant vesicular stomatitis virus is one of the most promising vaccine platforms, more so against the Zaire Ebola virus [69]. The vaccine is based on attenuated, replication-competent, recombinant stomatitis virus and is genetically engineered to express ZEBOV-GP as the immunogen [69]. Since the vaccine vector is attenuated, the immunized subjects only experience transient vector viremia [69]. Extensive rodents [43] and nonhuman primate trials demonstrated the protective potential of rVSV-ZEBOV-GP against the Zaire Ebola virus [[29],[43]]. To that extend, important immunogenicity, safety, and efficacy information have been provided by clinical trials and the ring vaccination of populations at high risk [[37],[69],[81],[94]]. In so far, the VSV-EBOV vaccine was demonstrated to be safe and successfully protects humans against the Zaire Ebola virus, as such the vaccine is in the process of being licensed for mass production and stocking [23].

Furthermore, the rVSV vaccine platform has so far been expanded to all known Ebola virus species [57]. In addition a blend of Ebola virus and Marburg virus in rVSV vaccine platform was able to confer protection against Ebola and Marburg virus challenge in nonhuman primates [28]. As such, there is need to investigate the likelihood of developing the platform into a single vector that will confer cross-protection against all known Ebola virus species.

Recombinant rhabdovirus-*based vaccine platform.* Interestingly, another milestone development is designing of a rhabdovirus vector that acts as a dual vaccine and can be used against Rabies and Zaire Ebola virus in high risk population of human and nonhuman primates [60]. The rabies virus-based vector was genetically engineered to reduce the neurovirulence of the rabies virus and to express ZEBOV-GP. The vaccine has been demonstrated to be highly immunogenic and conferred protection against rabies and Zaire Ebola virus challenge in rodent [64] and in Rhesus macaque models [5]. These results are quite encouraging towards the development of a dual vaccine for rabies and Zaire Ebola virus and can be used in a high-risk target population in African.

The efforts put in place for the development of Ebola vaccine is commendable. However, implementation of experimental trials of Ebola vaccines programs have faced a lot of challenges [24]. Such challenge include getting the appropriate population due to fear of the vaccine causing an infection [[18],[52]]. As such, many authors' suggests that in experimental intervention health workers should be given priority in following experimental intervention as sighted by Folayan et al. [24].

Development of Ebola antiviral drug

It is widely accepted that to date, there are no chemotherapeutic options available to treat Ebola disease. However, advances in research have developed four experimental drugs against Ebola virus disease, of which two of them have shown promising results. These drugs include REGN-EB3, mAb114, remdesivir, and ZMapp, all of which are a combination of monoclonal antibodies [[61],[94]]. Following clinical trials safety, remdesivir and ZMapp were dropped since they were less effective at preventing death. On the other hand, the patients on REGN-EB3 and mAb114 had more than 70% chance of survival [61]. A major disadvantage in development of anti-Ebola drugs is that, there has been pressure to deploy trial drugs that have shown promising results in the laboratory but lack supporting data from clinical trials to prove their safety and efficacy amid large-scale epidemics. Further, however promising these drugs are, effective treatments alone may not end Ebola outbreaks. As such, there is a need to design more safe and efficacious broad-spectrum drugs that will be effective against all the known Ebola species.

Conclusions and future direction

Ebola virus disease is arguably considered as one of the highly severe and fatal diseases of primates with great economic losses. Lack of access to rapid diagnostic tools has proven to be a setback to early identification, isolation, and management of the disease. This is further complicated by the non-specificity of symptoms presented during early stages of Ebola disease infection With such nonspecific symptoms and the virus being highly contagious with high mortality rate, the need importance of developing a rapid diagnostic assays that can be deployed to resource-limited regions to be used at the point of care for diagnosis and identification of the disease cannot be overemphasized.

Ebola virus disease has emerged as the most significant global health challenge due to multiple disease outbreaks for the last 3 decades. Recent advances in the development of effective Ebola virus vaccine and antiviral drugs have shown great promise. However, lack of vaccines, specific diagnostic tests, rapid geographical dissemination, and non-specific clinical signs pose a major challenge in combating the dreaded Ebola virus. Furthermore, however promising the current candidate vaccines are, they are monovalent vaccines, mostly against Zaire Ebola virus. Monovalent vaccines may take time to be deployed and cannot be used for prophylactic protection because, first the strain has to be identified then the appropriate vaccine deployed. Considering the highly variable *Ebolavirus* species and the high morbidity and mortality of associated with the infection, this will lead to fatalities that would have otherwise been avoided. Therefore, the need for a broadly applicable multivalent or polyvalent prophylactic vaccine against all the known species of Ebola virus cannot be over-emphasized. A prophylactic vaccine can be developed through prediction and elucidation of novel viral epitope conserved across all the members of genus *Ebolavirus*. Although it has been shown that there is potential in developing a prophylactic protection against different filovirus species by including multiple glycoprotein antigens in multivalent vaccines [58], a major challenge becomes identification of antigens conserved across all the species of *Ebolavirus* and that are capable to induce adaptive immune response in humans. Therefore, this calls for advances in designing a novel polyvalent vaccine and a broad-spectrum anti-Ebola virus drug that will be effective against all the known Ebola virus species.

Following the most devastating Ebola pandemic of (2014–2016), efforts toward EVD treatment continue to inspire scientific curiosity. Indeed, EVD pandemics has highlighted our awareness of the need to develop an efficacious treatment option. Consequently, numerous countermeasures have been developed including vaccines (rVSV-ZEBOV and Ad26-ZEBOV/MVA-BN-Filo prime-boost vaccine), nucleoside and nucleotide analogues (BCX4430, favipiravir, and GS-5734), plasma transfusions (Ebola-Tx), immunotherapeutics (Zmapp and MIL77), nucleic acidbased drugs, and repurposed drugs. There is need to widen the scope in the development of therapeutic agents with broad-spectrum activity against filoviruses like Marburg virus, Sudan virus, or other viral pathogens. Regrettably, drug discovery pipeline lead to only a small number of compounds that enter clinical trials, making it a challenging exercise because, largely, drug development has relied heavily on animal models of EVD. It is imperative that pharmaceutical companies explore In-silico drug discovery methods which are becoming increasingly feasible options of drug design and development. MD simulations can make useful contributions in understanding the molecular mechanisms underlying disease pathogenesis. There is enormous amount of data readily available which could be explored using computational approaches. With advancement in understanding the mechanism and mode of action of EBOV, future in silico processes remains key in the development of drug candidates against the devastating EVD.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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